FURTHER STUDIES ON THE EFFECTS OF DESICCATION OF THE VIRUS OF RABIES, AND THE USE OF THIS MATERIAL IN IMMUNIZATION.*†

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Harris and Shackell,[1] using the method described by one of them,[2] were able to preserve for several months some of the infectivity of rabies virus by drying this *in vacuo* at the temperature of salt and ice (−18° C.) Harris[3] modified this by freezing the material with CO_2 snow or liquid air, and then drying the virus *in vacuo* as before. Prepared in this manner, the rabic material maintains a large percentage of its infectivity for a comparatively long time. This paper records further observations on the rate at which infectivity of desiccated virus is lost, and on the use of this material for antirabic immunization.

In the method employed, the rabic brain and cord is frozen with CO_2 snow, pulverized in a mortar and dried *in vacuo* over sulfuric acid at a temperature of −15° to −18° C. The material thus dried is sealed free from all moisture in glass tubes and kept in an ordinary ice-box (8–12° C.).

For comparison of the infectivity of this material with that of cords dried by Pasteur's method, I have adopted the figures obtained by Harvey and McKendrick,[4] who, in a large series of experiments, carefully determined the infectivity of rabic cords when dried over KOH at 23° C. to be as follows: The minimal infective dose of fresh cord injected subdurally into rabbits is 0.025 mg. Of the "one day" cord the M.I.D. is twice that amount (0.05 mg.); of the "two day" cord, 8 times (0.20 mg.); of the "three day" cord, 40 times (1.0 mg.); and of the "five

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[1] *Jour. Infect. Dis.*, 1911, 8, p. 47.

[2] Shackell, *Am. Jour. Physiol.*, 1909, 24, p. 325.

[3] *Jour. Infect. Dis.*, 1912, 10, p. 369; *Ann. de l'Inst. Pasteur*, 1912, 26, p. 372.

[4] *Theory and Practice of Anti-rabic Immunity*, Calcutta, 1907, p. 13.

day" cord, 80 times (2.0 mg.). The "nine day" cord is practically non-infective.

A curve showing this rate of loss has been plotted by these authors to demonstrate more clearly the rapid decline in infectivity during the first three days and the more gradual subsidence after the fifth day. This curve is practically identical with one representing the loss of water from day to day, and these authors conclude that "the rate of loss of infectivity of rabies cord undergoing slow desiccation is directly proportional to the rate of loss of water contained in that cord," and that "they represent two parallel effects of the same causal action." Harris and Shackell accept this interpretation, and state, by way of explanation; "It is the general belief that the attenuation of a rabic cord depends primarily upon the loss of water. Our work leads us to believe that it is the *method* of extracting the water which results in attenuation or destruction of virulence and not the extraction of water *per se*. To state it differently, slow desiccation attenuates and destroys the virus directly by reason of the concentration of salts and other substances which are in solution in the brain and cord. The action is therefore, in essence, a chemical one.

In a series of tests made upon desiccated brain and cord, I have found that 0.004 mg., when injected intracranially, within one to five days after drying will infect rabbits and produce paresis by the sixth or seventh day. Allowing for a loss in weight of 75 per cent, due to the dehydration, this quantity would be equivalent in weight to 0.016 mg. of fresh undried material. The M.I.D. is therefore about two-thirds that of fresh cord. The material, kept in sealed moisture-free tubes, loses one-half its infectivity after 21 days. In other words, the M.I.D. is then 0.008 mg. After 50 days it is 0.01 mg.; after 100 days, 0.02 mg.; after 200 days, 0.05 mg.; after 500 days, 0.1 mg.

In Chart 1 a curve is plotted upon the results of the tests. The vertical line represents the amount of infective units in a milligram of dried material; the horizontal corresponds to the number of days of desiccation (temperature 8–12° C.). The curve shows the number of infective units contained in a milligram of desiccated material at any given time.

Table 1 gives some of the details of the experiments upon which this curve is calculated. All material used in these experiments had been preserved in sealed glass tubes, and placed in an ordinary refrigerator (8–12° C.). Series 190 and 193 were sealed at atmospheric pressure. The others were sealed *in vacuo*.

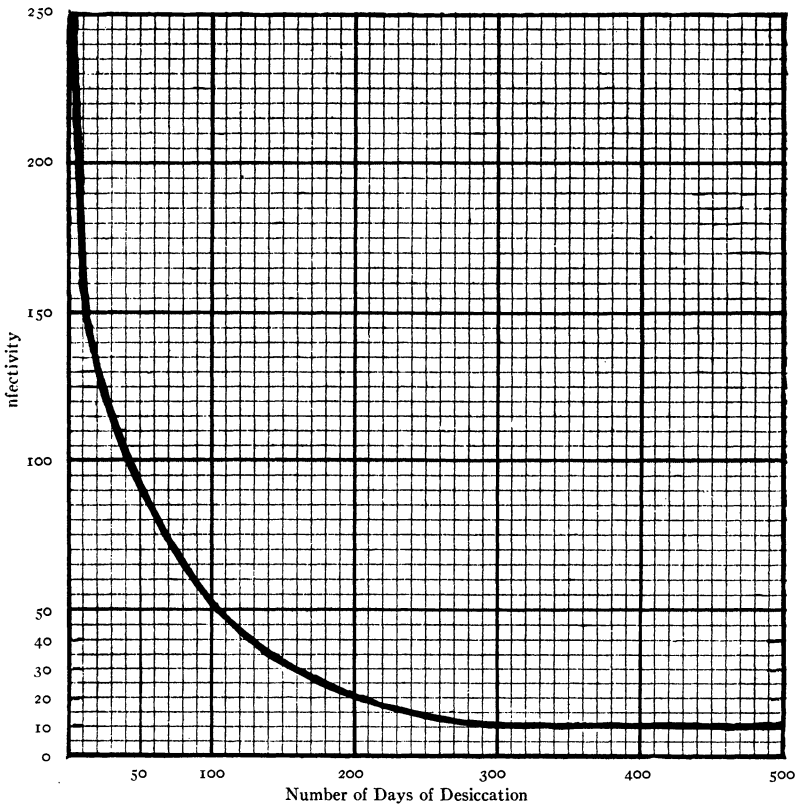

CHART 1.—Showing the rate at which infectivity of desiccated material is lost when preserved at a temperature of 8° to 12° C. The curved line indicates the number of M.I.D. in a milligram of material after the various periods of preservation shown in the base line.

In making the inoculations the usual practice is to emulsify 10 mg. of the material in salt solution which is added in small quantities from time to time up to 10 c.c. This gives a dilution of 1 mg. to each cubic centimeter of emulsion and from this other dilutions are made as desired. One-tenth (1/10) c.c. of the dilution is injected *into the brain* of a rabbit by trephining and passing a

TABLE 1.

Date	Series	Age of Material Days	No. of Rabbits	Amount Injected	Results
11-10-11....	184	15	6	0.02, 0.02, 0.04, 0.04, 0.2, 0.2 mg.	All paretic on the 6th day.
2-5-12.....	184	97	4	(A) 0.01, (B) 0.02, (C) 0.05, (D) 0.1 mg.	Rabbits C and D paretic on 6th day. A and B survived.
3-21-12....	184	142	3	(A) 0.1, (B) 0.2, (C) 1.0 mg.	A and C paretic on 6th day. B survived.
5-8-12.....	184	190	4	0.1, 0.1, 0.33, 0.5 mg.	All rabbits paretic on 6th day.
8-28-12....	184	302	2	0.1, 0.125 mg.	Both rabbits paretic on 7th day.
3-20-13....	184	502	3	0.1, 0.1, 0.15 mg.	All showed symptoms on 7th day. Paretic on 8th day.
2-22-12....	*190	2	3	(A) 0.005, (B) 0.01, (C) 0.02 mg.	Rabbits A and C ataxic on 6th day. B survived.
4-10-12....	190	50	1	0.01 mg.	Paretic on 6th day.
4-18-12....	190	58	3	(A) 0.0125, (B) 0.02, (C) 0.1 mg.	A and C ataxic on 6th day. B survived.
12-13-12....	190	295	2	0.01, 0.02 mg.	Both showed symptoms on 7th day.
3-13-12....	*193	5	5	0.0005, 0.001, 0.001, 0.002, 0.004 mg.	The rabbit injected with 0.004 paretic on 7th day. The rest survived without symptoms.
4-11-12....	193	33	4	0.005, 0.005, 0.01, 0.01 mg.	One rabbit injected with 0.01 mg. paretic on 9th day. Rest survived.
5-31-12....	193	83	3	0.02 mg. each	All paretic on 7th day. *This material, nos. 190 and 193, was preserved in tubes sealed at atmospheric pressure.
11-26-12....	214	31	2	0.01 mg. each	Both paretic on 7th day.
2-28-13....	214	125	2	0.02 mg. each	One paretic on 7th day. No symptoms in other.
10-26-12.. .	213	48	4	0.0166, 0.02, 0.025, 0.033 mg.	All four paretic on 6th day.
3-28-13....	213	201	2	0.05 mg. each	Both paretic on 6th day.
5-16-12....	202	26	3	(A) 0.01, (B) 0.02, (C) 0.02 mg.	A and C paretic on 6th and 7th day respectively. B survived.
12-26-11....	185	31	5	0.01, 0.02, 0.02, 0.05, 2.0 mg.	All five paretic on 6th day.
5-17-12....	198	27	3	0.0133, 0.02, 0.15 mg.	All 3 paretic on 6th day.
2-24-12.. .	187	45	2	(A) 0.01, (B) 0.02 mg.	B paretic on 6th day. A survived.
1-22-12....	185	56	3	(A) 0.005, (B) 0.01, (C) 0.02 mg.	C paretic on 6th day. A and B survived.
12-24-12....	217	1	3	0.004, 0.005, 0.005 mg.	Symptoms on 6th day. Paresis in all on 7th day.
1-15-13....	217	22	3	0.008, 0.01, 0.01 mg.	All paretic on 7th day.
1-24-13..	217	32	1	0.01 mg.	Paresis on 6th day.
2-12-13....	217	51	2	0.01, 0.0125 mg.	Both rabbits paretic on 6th day.

very fine hypodermic needle through the brain to the base, or if preferred into the lateral ventricle. With this method none of the material injected escapes after the needle is withdrawn, an occurrence which is liable to happen when the injections are made under the dura.

These tests indicate that my material, after a preservation for three weeks, with due allowance made for loss in weight, has an

infectivity equivalent to that of fresh cord; after 50 days it is 25 per cent more infective than the same quantity of the "one day" cord. After 200 days its infectivity is exactly equal to that of the "two day" cord. When kept 500 days it is two and one-half times as infective as the "three day" cord. In Chart 2 these relative values are illustrated diagrammatically.

The experiments detailed above were made upon material which had been kept in tubes *in vacuo*. A second set of tests (see Table 1, series 190 and 193) were made upon material kept in tubes sealed at atmospheric pressure. Extreme care must be taken in preparing these tubes to avoid the presence of any moisture. There does not appear to be any difference in the rate of loss whether it be preserved *in vacuo* or in dry air. When, however, there is the slightest moisture present infectivity is rapidly destroyed.

The rate of loss is greatly altered by variation of the temperature at which the material is preserved. At room temperature infectivity rarely lasts longer than five months. On the other hand, the lower the temperature, the slower the loss. One lot was kept at $-1°$ to $-3°$ C., and tests of its infectivity made after 32 and 75 days. Five rabbits were injected with 0.004 mg. each, and all were paretic by the seventh day. During this period there was therefore no appreciable loss in infectivity. It will be recalled that material kept for this length of time at 8–12° C. loses more than three-fifths of its infectivity.

During the past 15 months 182 patients have been injected with this material for the prevention of hydrophobia. It has also been used on a large number of dogs which had been either bitten by or in contact with rabid dogs. No deaths have occurred in either the patients or the animals, and no complications have developed. Of these patients, 15 had been bitten on the face by rabid dogs, 59 on the hand, 55 on other parts of the body, while 53 had been licked on the hand or face by rabid dogs.

As this material differs considerably from the cords used in the Pasteur scheme, it became necessary, in adopting it for immunizing purposes, to follow the work of Höyges, whose method is based upon the dilution of virus rather than upon quantitative destruc-

tion in drying. Calculations show that Höyges injected in mild cases an amount equal to 54.575 mg. of fresh cord. In severe cases he gave 97.47 mg. In terms of infective units, the latter represents 3,898 M.I.D., the former, 2,025 M.I.D. According to

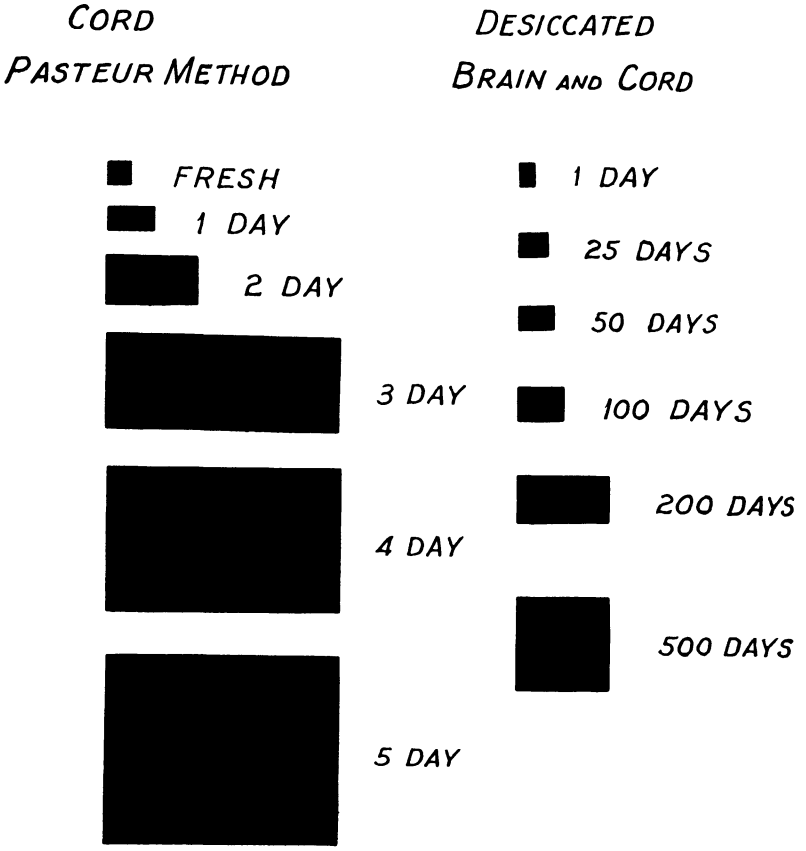

CHART 2.—Illustrating the comparative amount of cord (Pasteur) and of desiccated brain and cord which, after various periods of preservation, must be injected to produce the disease in rabbits. The M.I.D. of fresh cord is 0.025 mg. After 5 days it is eighty times this (2.0 mg.). When brain and cord are desiccated the M.I.D. is 0.033 mg. After 500 days, the M.I.D. is 0.4 mg. Thus one milligram of desiccated material 500 days old is five times as infective as one milligram of a 5-day cord.

the figures given by Harvey and McKendrick, the equivalent of the Pasteur scheme is 2,160 M.I.D.

With this as a basis, there remained the question of the quantity to be given daily and the total amount. In the light of our later knowledge concerning vaccinal immunity, I deem it unnecessary

gradually to increase the dosage to a certain point and then decrease, with alternating increase and decrease for a period of two or three weeks. I therefore have steadily increased the number of "units" (i.e., minimal infective dose) daily for the first three days and repeated this maximum quantity until a sufficient amount has been given. This total quantity depends entirely upon the location and extent of the injury, that is, upon the degree of probability of the development of hydrophobia. I also decided in mild cases to give as a minimum 5,000 M.I.D. or "units" distributed as follows: first day, 250 units; second day, 500 units; third day, 1,000 units; fourth day, 1,000 units; fifth day, 1,000 units; sixth day, 1,250 units. In ordinary cases of wounds on the hand, I give 10,000 units in 8 days, and in cases of extensive multiple, lacerated wounds of the face, I have given 30,000 units in a period of 15 days.

The question of the danger of the administration of so many infective "units" in so short a time has to be considered. In the many thousands of patients who have received anti-rabic treatment, there are very few recorded cases of infection by the fixed virus, and, though this danger is extremely remote, it has to be taken into account. In over 10,000 patients treated by the Höyges method, no such infection has been recorded. In that method, from 70 to 220 M.I.D. are injected the first day and from 200 to 400 M.I.D. the second.

Proescher and Ferran begin with comparatively large doses of fresh untreated virus. In a series of experiments Babes[1] showed that while the subcutaneous injection of fresh virus into dogs is followed occasionally by rabies, this danger can be obviated by the preliminary injection of a five-day cord. He also states that the injection of a large dose is less likely to infect animals than a small dose. The rational explanation for this apparent safety in an attenuated cord seems to be that the partially dried material contains the virus in a state so far injured as to be incapable of infecting, and yet capable of producing antibodies and a certain degree of immunity. This work of Babes led me to employ for the preliminary doses material which contains a very small amount

[1] *Traité de la rage*, Paris, 1912.

of infective units and a relatively large amount of altered or non-infective virus.

Chart 3 illustrates the relative amount of infective and non-infective material in a milligram of desiccated virus at various periods. Beginning with a maximum infectivity of one day, it will be seen that in 25 days, one-half of this has become non-infective; after 50 days, three-fifths are non-infective; after 100 days, four-fifths are non-infective; after 200 days, twenty-three-twenty-fifths. After 300 days only one-twenty-fifth of the original infectivity remains. When, therefore, one milligram of this last

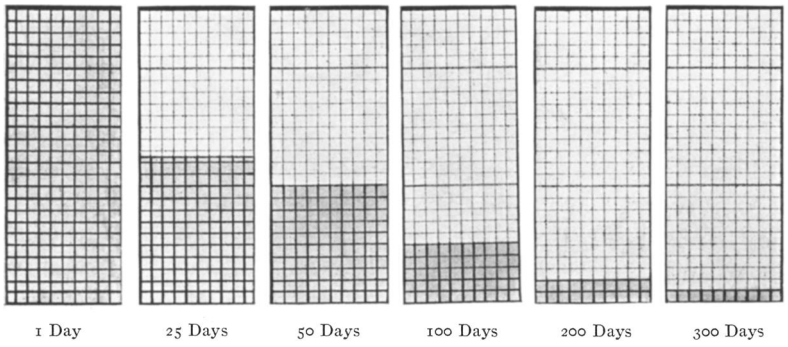

CHART 3.—Showing the relative proportion of infectious to non-infectious material in a milligram of desiccated brain and cord after preservation for various periods. The infectious portion is shaded. The number of shaded squares represents the number of "units" at any given time.

material is injected, the patient receives 10 infective units and 240 units which have become non-infective.

Several investigators have shown that after the Pasteur cords have lost all infectivity, they are but very slightly capable of producing an immunity. Harvey and McKendrick[1] state: "The immunizing power of a given portion of rabies cord is a function of the unkilled remnant of rabies virus which is contained in that cord." There is, according to these authors, no intermediate status between a "living," or immunizing, and a "dead," or nonimmunizing, state. It is well known, however, that other methods than drying may be used to destroy the infectivity of rabies virus without destroying its immunizing qualities. Babes used heat and

[1] *Loc. cit.*, p. 16.

Fermi uses carbolic acid for this purpose with excellent results. It is generally believed that, for the artificial production of active immunity, the injection of living organisms confers the greatest and most lasting immunity. In the preparation of bacterial vaccines, every agent used to kill the bacteria destroys to a certain degree the immunizing capacity of the product. Therefore, the best and safest method for the preparation of vaccines will be the one which destroys infectivity with the least degree of alteration of the antigenic substances.

Experiments seem to show that that portion of desiccated virus which becomes non-infective as time passes still possesses most of its immunizing properties. I have already shown[1] that when all the infectivity of this material has been destroyed with comparative rapidity by light and a higher temperature, it is still capable of rapidly conferring a high degree of immunity when injected subdurally. In other experiments I have conferred adequate immunity to dogs by the injection of a few doses of material which was several months old, and which contained only a very small proportion of the original infectivity.

The immunizing value of that portion of this material which has become non-infective cannot be determined with exactness until such a time has elapsed that all infectivity is lost. At present the oldest supply has been in the ice-box 500 days, and still infects rabbits in a dose of one-tenth milligram.

For the reason given above, my practice is to begin treatment with material in which the proportion of living to non-infection is 1 to 25; that is, material at least 6 months old. As the treatment continues, a gradual increase is made until material which contains 100 units per milligram is used. The fact that no accidents have occurred during the past 2 years in either patients, dogs, or rabbits is evidence of the safety and efficiency of the method.

The immunization by means of this material combines the advantages possessed by the Pasteur and the Höyges schemes. It combines the use of the attenuated and non-infectious material of the former with the simplicity of the quantitative dilution of the latter.

[1] *Jour. Infect. Dis.*, 1912, 11, p. 397.

If, as seems well established, the degree of immunity depends upon the amount of infective units injected, this method should give a greater degree of security than either the Pasteur or the Höyges scheme, since I administer from 2 to 10 times as many units to each patient as is ordinarily given.

The advantages claimed for this method of preparation are safety, economy, and convenience. It is safe because the greater portion of the material injected is capable of immunizing without being infectious. It is economical both to the patient and to the laboratory because it requires a much shorter time to administer a full treatment than most of the older methods. It is ofttimes a great hardship to require persons to leave home for a period of three weeks or more, not to mention the added cost to them for board and lodging.

It is especially economical to the laboratory in time, labor, and money. Material can be prepared two or three times a year and put aside in the cold to be used only when needed; and as one rabbit furnishes enough material to immunize from 20 to 25 patients, the initial cost of this is practically negligible. The work can be undertaken in any hospital or municipal laboratory without increasing the staff or the expense. To be able to prepare at one time material enough for from 6 to 12 months' use, and to have this always ready for any number of patients, is such a lessening of labor and anxiety as only those who have followed the classic method of drying cords can appreciate.